A6.14  
**mTOR DIRECTED MESENCHYMAL TISSUE RESPONSE TO INFLAMMATION IN ARTHRITIS**

Karonitsch, T; Dalwigk, K; Glehr, M; Niederreiter, B; Smolen, JS; Steiner, CW; DISH.

**Background** Accumulating evidence supports the concept that fibroblast-like synovocytes (FLS) actively participate in the destructive, inflammatory process of rheumatoid synovitis. Thus, FLS frame a synovial microenvironment that augments and perpetuates synovial inflammation. Moreover, FLS, together with macrophages form an aggressive mass of cells ("pannus"), which invades and destroys the articular cartilage. The mechanistic target of rapamycin (mTOR) is best known for coupling energy and nutrient abundance to the execution of essential cellular processes, including cell growth and cell survival. More recent data indicate that mTOR directs the cellular response to inflammatory stimuli in cells of the immune system. It remains elusive, however, whether or not this also applies to mesenchymal cells, such as FLS in the context of rheumatoid synovitis.

**Materials and Methods** In order to assess mTOR activity by immunohistochemistry (IHC) as well as western blotting (WB), phosphospecific antibodies against mTOR (IHC) and mTOR substrates, including 4E-BP (IHC), AKT (WB), S6K1 (WB), and S6 (IHC) were used. To determine the functional significance of mTOR activity in FLS, Torin-1, a well defined, specific inhibitor of mTOR, was used. To establish a role for mTOR in the mesenchymal, inflammatory tissue response, we used a previously described simplified 3-D model of the synovial tissue. IL-6 and IL-8 levels in the supernatants of 3-D cultures were measured by ELISA.

**Results** mTOR, 4E-BP and S6 were found to be phosphorylated in RA synovial tissues. These activated phospho-proteins were preferentially expressed in FLS, most prominently in the hyperplastic synovial lining layer. In-vitro, TNF stimulation of FLS resulted in the phosphorylation of AKT and S6K1, indicating that TNF activates the mTOR pathway in FLS. Stimulation of the 3D cultures with TNF resulted in hyperplasia of the lining layer at the surface of the spheres. Strikingly, treatment with Torin-1, prevented TNF induced lining layer hyperplasia. Unexpectedly, the combined treatment of 3-D cultures with TNF and Torin-1 resulted in increased production of IL-6 as well as IL-8 when compared to cultures that were solely exposed to TNF.

**Conclusions** These studies provide insight into the regulatory circuits that determine the synovial mesenchymal tissue response to inflammation and suggest a multifaceted role for mTOR in arthritis.

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**A6.15  
REFRACTORY CHRONIC ERYTHEMA NODOSUM AND TREATMENT WITH ANTI TNF**

Uceda, J; Hernandez, R; Mayordomo, I; Marenco, JL. Rheumatology Department, Valme University Hospital, Seville, Spain

**Introduction** Erythema nodosum septal panniculitis without vasculitis, characterised by acute episodes of inflammatory and painful subcutaneous nodules affecting, in most cases, the lower extremities. Remission of lesions occurs within 1–6 weeks without scarring or residual atrophy. There are, however, some cases which become chronic or recurr. In 50% of cases we find an underlying cause. Treatment of acute outbreak involves rest and NSAIDs. The chronic or recurrent cases are treated with oral potassium iodide, corticosteroids, colchicine, hydroxychloroquine or immunosuppressive agents.

**Objectives** Description of the cases of refractory chronic erythema nodosum and review of the literature.

**Methods** Selection of patients with refractory chronic erythema nodosum undergoing treatment with anti TNF in the Rheumatology unit from 2000 to 2010. Literature search using PubMed with keywords erythema nodosum and Adalimumab, Etanercept, Infliximab.

**Results** See table 1.

**Conclusions** In our sample, all cases have responded favourably to treatment with anti TNF. No adverse events were observed, except the occurrence of cutaneous psoriasis in one patient after...
infliximab treatment. In reviewing the literature we find that anti-TNF paradoxically brings about an immediate response in erythema nodosum patients, however provokes erythema nodosum and other skin manifestations in patients with either rheumatic pathology or inflammatory bowel disease. [1, 2]

Bibliography

7. Genetics and epigenetics of rheumatic diseases

**A7.1**

A GENETIC VARIANT IN THE REGION OF MMP-9 IS ASSOCIATED WITH SERUM LEVELS AND PROGRESSION OF JOINT DAMAGE IN RHEUMATOID ARTHRITIS

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1DPC de Rooy, A’ Zhemakova, RI Tsonaka, A’ Willemze, BAS Kurkmeerman, R’EM Toes.
2TWU Huizinga, JJ Houing-Duistermaat, PK Gregersen, AHV van der Helm-van Mil.
3Department of Rheumatology, Leiden University Medical Center, Leiden, The Netherlands;
4Department of Medical Statistics, Leiden University Medical Center, Leiden, The Netherlands;
5Feinstein Institute for Medical Research and North Shore–Long Island Jewish Health System, Manhasset, New York, USA

Background and Objectives The severity of joint destruction is highly variable between Rheumatoid Arthritis (RA) patients. We aimed to identify new genetic risk factors by studying genetic susceptibility loci of several auto-immune diseases.

Patients and Methods In phase-1, 646 Dutch RA-patients with yearly X-rays of hands and feet over 7 years follow-up were genotyped for 148,880 SNPs by Immunochip which contains 186 loci previously associated with autoimmune diseases. Association of SNPs with MAF > 0.01 (130,841 SNPs) with joint destruction was analysed using a marginal regression model. Correction for multiple testing was done by Bonferroni correction for the number of uncorrelated SNPs (threshold p < 1.1 x 10^-6). In phase-2, 686 North American RA-patients with repeated hands X-rays over 15 years follow-up, for which Immunochip genotyping data were available, were studied. SNPs that were significantly associated in phase-1 were selected and evaluated. All X-rays were scored by Sharp van der Heijde score (ICC 0.91 and 0.98 for phase-1 and 2 respectively). MMP-9 levels were measured in baseline serum by ELISA (Ebioscience) in 120 RA-patients that were selected on the Rs11908352-genotype.

Results In phase-1, 109 SNPs were significantly associated with joint destruction (p < 1.1 x 10^-6). Of these, 76 variants were on the HLA region. The 33 non-HLA genetic variants, though several were in high LD, were studied in the North-American RA-patients. Here, after correction for the number of uncorrelated SNPs (threshold p < 0.0036), two variants were associated with the severity of joint destruction: Rs451066 on chromosome 14 (p = 0.002, MAF = 0.20) and Rs11908352 on chromosome 20 (p = 0.002, MAF = 0.21). The region of Rs451066 on chromosome 14 has previously been linked to type-1 diabetes susceptibility. Presence of a risk allele was associated with a 5.7% higher rate of joint destruction per year; this equaled 29% over 7-years. Rs11908352 is located at the MMP-9 region on chromosome 20. Patients with a risk allele had a 2.7% higher radiological progression rate per year, which equaled 20% more joint destruction over a 7-years period. Furthermore, the minor genotype was associated with significantly higher levels of MMP-9 compared to the common genotype (p = 0.007).

Conclusions Two new risk loci for progressive joint destruction in RA were identified (Rs451066 and Rs11908352). The risk allele in Rs11908532 also associated with higher serum MMP-9 levels, indicating to a role for MMP-9 in progression of joint destruction in RA.

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**A7.2**

ALLOGRAFT INFLAMMATORY FACTOR 1 (AIF1) POLYMORPHISMS IN FRENCH CAUCASIANS WITH RHEUMATOID ARTHRITIS

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1Doua F Azzouz, 1Nathalie Balandraud, 1Sami B Kanaan, 1Isabelle Augier, 1Marielle Martin, 1Fanny Amoux, 1Jean Roudier, 2Nathalie C Lambert. 1INSERM UMRs 1097, Marseille; 2Rheumatology Department, Ste Marguerite Hospital, AP-HM, Marseille

Background Allograft inflammatory factor 1 (AIF1) is a cytoplasmic inflammatory protein encoded within the HLA class III genomic region on chromosome 6 (6p21.3). Although several risk loci for Rheumatoid Arthritis (RA) have been identified by Genome Wide Association Studies (GWAS), none of them involved AIF1 polymorphisms. However, two studies on small cohorts have shown that AIF1 single nucleotide polymorphism (SNP) Rs2269475 (G/T), causing a non-synonymous change of amino acid, is associated with RA (Harney, SM et al, 2008; Pavlik A et al, 2005). Moreover, AIF1 overexpression in inflammatory synovial tissues and macrophages isolated from synovial fluids of patients with RA, confirms its potential role in RA.

Objective We propose to examine the association of the seven most described AIF1 SNPs in our French RA patients.

Methods We have tested 99 Anti-Citrullinated Protein Antibody (ACPA) positive Caucasian RA patients who fulfilled ACR/EULAR criteria and 104 healthy Caucasians. We designed AIF1 primers to specifically amplify the AIF1 gene region containing the 7 SNPs: Rs2844475, Rs4711274, Rs2736182, Rs2736181, Rs2259571, Rs2269475 and Rs13195276. PCR products were sequenced (Cogenics Beckman Coulter) and chromatogram results analysed for the 7 SNPs positions in patients and controls. Patients and controls were genotyped for HLA-DRB1.

Results Two SNPs out of the 7 were associated with RA: Rs4711274 (G/A) and Rs2269475 (G/T). Regarding Rs4711274, G/A and A/A genotypes were increased when compared with controls (p = 0.0005). The minor A allele was strongly associated with RA (p = 0.0005). Regarding Rs2269475, in linkage disequilibrium with the former, we found a similar pattern with increased T/T and C/T genotypes (p = 0.0009) and increased minor T allele frequency (p = 0.0008) in patients with RA. Interestingly, patients carrying the minor associated AIF1 allele expressed HLA-DRB1*04 more often than the patient’s group carrying the C/C or G/G genotype (63.8% versus 44.4%), although the difference was marginal (p = 0.06).

Conclusions In this study of French Caucasians with RA, we confirmed Rs2269475 association already described in British and Polish Caucasians. Additionally, we find an association with Rs4711274 in linkage disequilibrium with Rs2269475. Intriguingly, such associations have never been found in GWAS.

**A7.3**

ASSOCIATION OF CIRCULATING MiR-223 AND MiR-16 WITH DISEASE ACTIVITY IN PATIENTS WITH EARLY RHEUMATOID ARTHRITIS

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1Mária Filková, 1Caroline Ospelt, 1Serena Vettori, 2Ladislav Šenolt, 2He Vencovský, 2Renate E Gay, 1Renate E Gay, 1Steffen Gay, 1Astrid Jüngel. 1INSERM UMRs 1097, Marseille; 2Rheumatology Department, Ste Marguerite Hospital, AP-HM, Marseille

Background MiR-223 and miR-16 are induced in rheumatoid arthritis (RA) synovium. We have tested 99 Anti-Citrullinated Protein Antibody (ACPA) positive Caucasian RA patients who fulfilled ACR/EULAR criteria and 104 healthy Caucasians. We designed AIF1 primers to specifically amplify the AIF1 gene region containing the 7 SNPs: Rs2844475, Rs4711274, Rs2736182, Rs2736181, Rs2259571, Rs2269475 and Rs13195276. PCR products were sequenced (Cogenics Beckman Coulter) and chromatogram results analysed for the 7 SNPs positions in patients and controls. Patients and controls were genotyped for HLA-DRB1.

Methods We have tested 99 Anti-Citrullinated Protein Antibody (ACPA) positive Caucasian RA patients who fulfilled ACR/EULAR criteria and 104 healthy Caucasians. We designed AIF1 primers to specifically amplify the AIF1 gene region containing the 7 SNPs: Rs2844475, Rs4711274, Rs2736182, Rs2736181, Rs2259571, Rs2269475 and Rs13195276. PCR products were sequenced (Cogenics Beckman Coulter) and chromatogram results analysed for the 7 SNPs positions in patients and controls. Patients and controls were genotyped for HLA-DRB1.

Results Two SNPs out of the 7 were associated with RA: Rs4711274 (G/A) and Rs2269475 (G/T). Regarding Rs4711274, G/A and A/A genotypes were increased when compared with controls (p = 0.0005). The minor A allele was strongly associated with RA (p = 0.0005). Regarding Rs2269475, in linkage disequilibrium with the former, we found a similar pattern with increased T/T and C/T genotypes (p = 0.0009) and increased minor T allele frequency (p = 0.0008) in patients with RA. Interestingly, patients carrying the minor associated AIF1 allele expressed HLA-DRB1*04 more often than the patient’s group carrying the C/C or G/G genotype (63.8% versus 44.4%), although the difference was marginal (p = 0.06).

Conclusions In this study of French Caucasians with RA, we confirmed Rs2269475 association already described in British and Polish Caucasians. Additionally, we find an association with Rs4711274 in linkage disequilibrium with Rs2269475. Intriguingly, such associations have never been found in GWAS.